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Serological responses to *Neospora caninum* in experimentally and naturally infected water buffaloes (*Bubalus bubalis*)

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Abstract

The water buffalo (*Bubalus bubalis*) is an important natural host for *Neospora caninum*. Serologic responses to *N. caninum* were studied in experimentally and naturally infected water buffaloes in Brazil. Antibodies were assayed by the indirect fluorescent antibody test (IFAT) using a cut off value of 1:25. Six buffaloes were each inoculated subcutaneously with 5×10^6 live culture-derived tachyzoites of the cattle Illinois strain of *N. caninum*, and two calves were kept as uninoculated controls. Post-inoculation (p.i.) blood samples were collected weekly for 8 weeks and then monthly until 1 year p.i. All inoculated buffaloes developed IFAT titers of 1:100 or more between 7 and 11 days p.i. and the titers remained elevated until 7 weeks p.i. Antibody titers peaked to 1:1600 in 1, 1:800 in 3 and 1:400 in 2, usually by 3 weeks p.i. Antibody titers declined to 1:25 or 1:50 in all the six buffaloes by 12 months p.i. IFAT titers to *N. caninum* remained at an undetectable level (<1:25) in both control uninoculated buffaloes. To follow the dynamics of *N. caninum* antibodies, sera from 29 buffaloes and their calves were collected for 1 year and assayed for *N. caninum* antibodies; 23 of 29 calves were seropositive (IFAT of 1:100 or more) at 1-2 day of age. Of these 23 calves, 17 remained seropositive during the study, while six became seronegative at four (two calves), six (one calf) seven (two calves) and eight (one calf) months of age. These findings suggest a high rate of neonatal transmission of *N. caninum* in buffaloes.
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Keywords: *Neospora caninum*; Water buffaloes; *Bubalus bubalis*; Congenital transmission; Antibodies; Experimental; Natural

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1. Introduction

Neospora caninum causes mortality in several species of animals including cattle, horses, and dogs (Dubey, 2003a). It is a major cause of abortion in cattle worldwide (Dubey, 2003b). Buffaloes are an important natural host for *N. caninum* (Rodrigues et al., 2004). Little is known of the persistence of maternally-acquired antibodies to *N. caninum* in ruminants. This information is vital for serological diagnosis of neosporosis. In the present paper, persistence of antibodies to *N. caninum* in 29 water buffalo *(Bubalus bubalis*) dams and their male calves from birth to 1 year of age are followed. In addition, the magnitude of serologic responses of calves inoculated with *N. caninum* tachyzoites was studied.

2. Material and methods

2.1. Experimental infections in buffalo calves

Eight buffaloes aged 8 months, with no demonstrable *N. caninum* antibodies by indirect fluorescent antibody test (IFAT) in 1:25 dilution of serum were selected for this study (Table 1). Six buffaloes were each inoculated subcutaneously with 5×10^6 live cultured derived tachyzoites of the cattle Illinois strain of *N. caninum* (Gondim et al., 2004), and two animals were kept as uninoculated controls. Post inoculation (p.i.) blood samples were collected weekly for 8 weeks, then monthly until 1 year p.i.

Table 1
N. caninum antibodies in young buffaloes experimentally inoculated with *N. caninum* tachyzoites

Collection period[a]	Buffaloes with *N. caninum* antibodies					
	I1	I2	I3	I4	I5	I6
D7[b]	<25	<25	<25	<25	<25	<25
D11	100	200	400	400	400	200
D14	800	400	800	400	800	400
W3[c]	1600	400	800	800	800	400
W6	1600	100	400	200	400	800
W8	400	50	400	200	25	100
W12	50	<25	50	25	25	<25
W24	50	50	50	50	50	50
W36	50	50	50	50	25	25
W48	25	50	100	25	<25	25

[a] Titers on days 0, and 3, and weeks 4, 5, 7, 8, 16, 20, 28, 32, 40, and 44 are not shown but they did not fluctuate more than one dilution.
[b] D = day.
[c] W = week.

2.2. Serologic responses in dams and their male calves

Sera from 29 female buffalo and their calves, 20 pairs were from two farms in city of Registro and nine pairs from Ilha Solteira city, both in state of São Paulo, Brazil were tested for IFAT antibodies. The samples were collected from 1 to 2 days after birth for 12 months after parturition (Table 2). It was not possible to collect sera from newborns animal before the colostral ingestion because of fear of rejection of the calves to their dams.

2.3. Serology

All sera were tested using indirect fluorescence antibody test (IFAT) to verify the presence of antibodies against *N. caninum,* as previously described by Fujii et al. (2001). The titer considered positive was the highest dilution able to determine fluorescence in whole tachyzoites and the cut-off value was 1:25. In each slide positive and negative buffaloes sera were included as control.

3. Results

3.1. Experimental infection

The six calves inoculated with *N. caninum* tachyzoites remained asymptomatic throughout the study but seroconverted between 7 and 11 days p.i. and the titers remained elevated until 7 weeks p.i. (Table 1). Antibody titers peaked to 1600 in one, 800 in three and 400 in two, usually by 3 weeks p.i. Antibody titers declined to 25 or 50 in all the six buffaloes by 12 months p.i. Antibody titers to *N. caninum* remained at an undetectable level (<25) in both control uninoculated buffaloes. Individual titers on selected intervals are shown in Table 1.

Table 2
N. caninum antibodies in buffaloes calves and their dams since birth to two for 12 months of age

Animals	Antibody titers												
	1 d	1 m	2 m	3 m	4 m	5 m	6 m	7 m	8 m	9 m	10 m	11 m	12 m
Farm A (Registro)													
Dam 1	100	100	50	50	n/c	100	50	100					
Calf 1	800	400	400	50	n/c	50	25	50	0	0	0	0	0
Dam 9	400	50	50	200	n/c	200							
Calf 9	400	800	400	200	200	50	25	25	25	25	25		
Dam 12	400	400	400	400	200	200							
Calf 12	800	800	400	200	400	100	100	100	50	50	100	100	200
Dam 17	800	400	400	400	800								
Calf 17	1600	1600	1600	800	400	200	50	200	200	50	50		
Farm B (Ilha Solteira)													
Dam 1	100	n/c	50	400	400	800	800	800	200	200			
Calf 1	1600	800	200	50	0	0	0	0	0	0	0	0	0
Dam 3	800	400	400	400	1600	1600	3200	1600	6400	12800	6400	6400	
Calf 3	1600	1600	1600	6400	3200	3200	n/c	3200	3200	3200	3200	800	
Dam 4	n/c	400	200	1600	800	800	n/c	800	1600				
Calf 4	1600	800	800	1600	1600	800	400	400	400	400	400		
Dam 9	1600	800	200	n/c	800	800	800	800	800	400	400	800	
Calf 9	6400	12800	400	100	0	0	0	0	0	0	0	0	

n/c: not collected. Collection days (d = days m = months). $0 \leq 25$.

3.2. Dam-calf serology

From 29 pairs of mothers and theirs calves, six of them were seronegative and remained so during the study. From 23 pairs that were seropositive at birth time, 17 remained positive during the entire study, while six turned seronegative at 4 (2), 6 (1), 7 (2) and 8 (1) months of age. From Registro city 14 pairs were positive at birth time, 10 maintained this condition during all time, and the other four pairs had negative serology at 6 (1), 7 (2) and 8 (1) months of age. From Ilha Solteira farms all the nine groups were positive at birth time. Two of nine young buffaloes turned negative by 4 months of age and remained negative until the end. The respective data from eight dam-calf pairs are shown in Table 2.

4. Discussion

In the present study, 23 of 29 (79%) of dams were seropositive at the time of birth, confirming a high prevalence of *N. caninum* antibodies in adult buffaloes in Brazil (Fujii et al., 2001). Of these 23 dam and calf pairs studied, antibodies persisted in 17 calves beyond 7 months, indicating neonatal acquisition of *N. caninum* infection. These calves either acquired persistent infection transplacentally or lactogenically. At present there is no evidence for lactogenic transfer of *N. caninum* infection (Davison et al., 2001). Finding persistent antibodies in 17 of 23 seropositive calves suggests a 74% congenital transfer rate of *N. caninum* in buffaloes. These findings are consistent with results obtained in cattle (Dubey, 2003a; Romero and Frankena, 2003). In the present study, colostrally-acquired antibodies persisted in some buffaloes for 7 months, which is a longer period than reported in cattle. Hietala and Thurmond (1999) found that passively acquired *N. caninum* antibodies disappeared in cattle by 180 days.

The results obtained in buffaloes inoculated with *N. caninum* tachyzoites were unexpected because antibodies in all six inoculated calves declined to 1:25 or 1:50 4 months p.i. Whether all these calves cleared *N. caninum* infection in unknown, but unlikely. The calves were inoculated with a recent *N. caninum* isolate of bovine origin and this isolate was capable of producing *N. caninum* oocysts (Gondim et al., 2004). The IFAT titer that should be considered indicative of a patent *N. caninum* infection in cattle or other animals is unknown. Venturini et al. (1999) suggested that an IFAT titer of 1:25 should be considered specific for *N.*

caninum and the results of the present study support this hypothesis because all pre-inoculation sera were negative at the 1:25 dilution of serum.

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